

Methods

¹H-NMR spectra of β -cyclodextrin (β -CD) complex was recorded in D₂O solution with a Varian spectrometer Mercury Plus (Varian Inc., Palo Alto, CA, USA) at 399.93 MHz. Chemical shifts are given in ppm (δ) which were measured relative to the peak of the solvent D₂O (4.65 ppm). All ¹H NMR spectra were recorded with a 5 mm tube in D₂O, without degassing.

¹H NMR spectroscopy has proved to be a good diagnostic tool as well as useful in the study and characterization of β CD inclusion complexes (references 1-3)

Because of the water solubility of all β CD-pyrethroid-PBO complexes at 20 °C at a reasonable concentration (>2 mM) is achieved, ¹H NMR experiments, performed in D₂O, provide further experimental evidence of the formation of these complexes since all pure pyrethroid (i.e. not complexed) are far soluble in water as reported in Table 1.

Table 1. Solubility of the pyrethroids in water (mg/l)

α -cypermethrin	0.01mg/l
biphenethrin	0.1mg/l
λ -cyalothrin	0.005mg/l
β -cifluthrin	2mg/l

Protons of the pyrethroid included in the β CD cavity appear sharp and clear whereas no detectable proton signals of the pyrethroid are observable in a mechanical mixture with β -CD and PBO either when we tried to use our procedure to prepare a complex with β CD and pyrethroid without PBO. In our procedure, the presence of PBO allows to achieve a formulate β CD-pyrethroid-PBO. According to ¹H NMR data on β -cyclodextrin complexes reported in the literature (1,2,3), chemical shifts of H³ and H⁵ β -CD protons, which point into the lipophilic cavity, are a useful probe to observe formation of inclusion complexes and, hence, to evaluate the structural modification of β -CD.

Chemical shift variations of H^3 and H^5 β -CD protons reflect the formation of a complex between them. In fact, the entry of the apolar guest into the lipophilic cavity of the host (β -CD) induces a shielding of H^3 and H^5 as reported in Table 2. Those data indicate that the pyrethroids are included into the lipophilic cavity of β -CD.

Although PBO have structural and electronical features to form a complex included in the cavity of β -CD, all pyrethroids (pyrethroids) examined form a more stable complex with the β -CD cavity compared to PBO. However according our statement PBO that is present in this complex is hence reasonably located in the hydrophilic part of β -CD.

The release time of pyrethroid was calculated by 1H NMR (recorded in D_2O) of the integration of the proton signals of the pyrethroid and β -CD.

Results

Table 2 shows the chemical shifts values for β -CD in the free and in the complex state measured in D_2O . The $\Delta\delta$ (ppm) represents the chemical shifts differences between the two states.

TABLE 2

	β CD Proton H^3 (ppm)	β CD Proton H^5 (ppm)	H^3 $\Delta\delta/H^5$ $\Delta\delta$ β CD free- β CD complex*	Release time at 22 °C, start release
β CD free	3.822	3.715	-----	
β CD - actp PBO complex	3.847	3.747	+0.025/+0.032	4 h
β CD - biphenthrin PBO complex	3.701	3.600	-0.120/-0.115	6h
β CD - λ -cyalothrin PBO complex	3.732	3.628	-0.020/-0.011	4h
β CD - β -ciflutrin PBO complex	3.866	3.800	+0.044/+0.085	5h

* - $\Delta\delta$: lower field; + $\Delta\delta$: higher field

By analysis of NMR spectroscopic data, has been found that the pyrethroid begins to be delivered by the complex only after some hours; on the contrary more than 90% of PBO can be released by the complex very quickly when it is dissolved/suspended in water, thereby maximising the synergistic effect.

In fact, it is interesting to observe in the ^1H NMR spectrum of formulate (recorded in D_2O) the presence of the same pattern of PBO protons (PBO state A) shifted to upfield (PBO state B) (Table 3). The amount of PBO state B is about 10% respect to PBO state A. It means that when the complex of the present invention is suspended in water more than 90% of PBO is immediately released, realizing our goal to introduce the better Δt (>4h) between the release of the two components (pyrethroid and synergic).

Table 3. Chemical shifts values in ppm for PBO in the state A and in the state B measured in D_2O

TABLE 3

	$\text{OCH}_2\text{O}_{\text{state A}}$ (proton, multiplicity)	OCH_2O (proton, multiplicity)	$\text{OCH}_2\text{O}_{\text{state B}}$ ppm	$\Delta\delta \text{OCH}_2\text{O}_{\text{state B}}$ and $\text{OCH}_2\text{O}_{\text{state A}}$
PBO Free	-----	5.465 (2H, s)	-----	-----
BCD-actp PBO complex	5.485 (2H, s)	-----	5.769 (2H, s)	+0.284
BCD-biphenanthrin PBO complex	5.360 (2H, s)	-----	5.660 (2H, s)	+0.300
BCD- λ -cyalothrin PBO complex	5.462 (2H, s)	-----	5.750 (2H, s)	+0.288
BCD- β -cyfluthrin PBO complex	5.449 (2H, s)	-----	5.750 (2H, s)	+0.201

References:

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- (3) Schneider, H.-J.; Hacket, F.; Rüdiger, V. NMR studies of cyclodextrins and cyclodextrin complexes. *Chem. Rev.* **1998**, 98, 1755-1785.